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Lethality after Intratracheal Challenge of SEB  
(Staphylococcal enterotoxin B) in Dutch Rabbits<sup>1,2</sup>

Ching-Tong Liu<sup>3</sup>, Donald A. Creasia, Dianne M. Walters,  
and Thomas J. Taylor<sup>4</sup>

Toxinology and Applied Research Divisions,  
U.S. Army Medical Research Institute of Infectious Diseases,  
Fort Detrick, Frederick, MD 21702-5011

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### Abstract

Selecting an animal species for studying SEB-induced toxicity has been difficult due to highly variable inter-species susceptibility. Approximately twenty years ago, non-human primates exposed intravenously or by inhalation of SEB were commonly used. Unfortunately, experiments with primates have been restricted because of limited toxin (SEB) supplies, as well as the high costs and animal use considerations for non-human primate studies. The purpose of this study was to develop techniques for inducing SEB toxicity from a pulmonary origin by direct intratracheal (IT) instillation in Dutch rabbits. Four doses of SEB (0.1, 0.3, 1.0, and 3.0 mg/kg, N = 3-9/group) were administered. Body weights and rectal temperatures were measured at 4-5 hr intervals after IT SEB injection for 40 and 80 hr, respectively. A trend of initially elevated rectal temperature over 20 hr was observed, and body weights decreased significantly 20 hr after IT SEB injection (0.3 - 3.0 mg/kg). The LD<sub>50</sub> was determined to be 0.46 mg/kg (N = 30) with 95% confidence limits (0.18 to 1.00 mg/kg). These results imply that: 1) the Dutch rabbit is acceptable for studying SEB kinetics after IT instillation, and 2) the IT SEB instilled rabbit may be appropriate for quantitative evaluations of immunological or/and pharmacological interventions during SEB toxemia, originated from a pulmonary route.

## Introduction

Staphylococcal enterotoxin B is produced by certain strains of Staphylococcus aureus (1-4). SEB-induced toxicity varies, depending on the route of exposure. In rhesus monkeys, oral ingestion of SEB induces vomiting and diarrhea, leading to loss of body fluids and electrolytes (5, 6). However, when a small amount of SEB is introduced into the lung as an aerosol (7, 8), or is injected intravenously (9-11), pulmonary edema and irreversible circulatory shock may develop, and death ensues.

The purpose of this study was to evaluate the feasibility and suitability of using direct IT SEB instillation for studying the toxicokinetics of SEB administered by a pulmonary exposure route (12-14). We previously demonstrated physiological changes and selected therapeutic responses after intramuscular administration of SEB in Dutch rabbits (15, 16). In this study, we developed simple techniques for direct IT injection of SEB into the lungs of Dutch rabbits under a short-acting gas anesthesia (3% isoflurane in oxygen). Because illness and dose-dependent lethality were induced after IT SEB challenge, experimental results support the notion that IT SEB instillation is acceptable for studying SEB transport from the lungs across alveolar and capillary membranes, appearing in the systemic circulation for toxicokinetics studies in Dutch rabbits.

## Materials and Methods

### 1. Animal groups:

Justifications of animal use and method of anesthesia were reviewed and approved by our Institutional Laboratory Animal Care and Use Committee. Dutch rabbits (Hazelton-HRP, Denver, PA), weighing 0.92 to 1.60 kg, were randomly allocated into control and four experimental groups injected IT with isotonic saline (control) or SEB in isotonic saline at doses of 0.1, 0.3, 1.0, and 3.0 mg/kg (N = 3-9/group). Rabbits were kept in an air-conditioned room at 20-21 °C, and were maintained in separate cages with water and food (Purina rabbit chow, Metro Feed, Columbia, MD) ad libitum.

### 2. Preparation of SEB solution:

Purified (>95%, lot No. 14-30) SEB (17) with endotoxin levels < 2.5 ng/mg protein, was prepared and provided by our Institute. Endotoxin was determined by a commercially available, plate-based chromogenic Limulus amebocyte lysate assay (Bio-Whittaker, Walkersville, MD). SEB was dissolved in isotonic saline, and its concentration was prepared as 0.2, 0.6, 2.0, and 6.0 mg/ml.

### 3. Anesthesia and intratracheal administration of SEB:

Dutch rabbits were anesthetized by 3% isoflurane-oxygen mixture with a non-rebreathing anesthesia vaporizer through a face mask (Summit Hill Laboratories, Navesink, NJ), operated at approximately 1 liter/min. The ventral aspect of the rabbit's neck was shaved and swabbed with 70% ethanol. The outline of the trachea under the skin was held steadily between the thumb and

forefinger of one hand, and a 23- gauge, 1-1/2 inch long needle attached to a 1.0 ml syringe was inserted at the level of tracheal rings 2 to 4 (Fig. 1). Injection volume of SEB was 0.5 ml/kg body weight for all doses. After the needle was inserted, tracheal air was aspirated to assure accurate tracheal penetration. The rabbit was held and inclined at approximately 45 degrees during and shortly after SEB injection, allowing the toxin solution to flow directly into the lungs. Rabbits recovered within 4-5 min from the gas anesthesia.

#### **4. Measurements of body weights and rectal temperatures:**

Body weights and rectal temperatures were measured before and after IT SEB administration at 4-5 hr intervals. The rabbit was weighed on a balance (Sartorius, type: LC34, Germany and Bohemia, NY). Rectal temperatures were measured with a glass mercury thermometer inserted approximately 4 cm into the rectum. Each animal was observed for signs of illness until death or recovery. At death, animals were examined to assess the site of SEB injection into the trachea.

#### **5. Statistical analyses:**

Based on lethality or survival data after an IT injection of SEB, a sigmoid lethality curve over various doses of SEB was produced. The median lethal dose ( $LD_{50}$ ) was calculated by using a PROBIT procedure (18). All other data analyses were also accomplished by using the SAS analysis program (18). Mean values with SEM are presented. Statistical significance was determined for body weight and percent of survival over time among five

groups of animals by using the GLM (general linear model) and LIFETEST programs, respectively. Differences were considered significant at  $P < 0.05$ .

## Results

Effects of various doses of IT SEB (0.1 - 3.0 mg/kg) on body weight changes over 40 hr in Dutch rabbits are shown in Fig. 2. Body weights remained unchanged in control animals and increased slightly in the experimental group receiving a low dose of SEB (0.1 mg/kg). Although rabbits receiving IT SEB at 0.3 mg/kg did not show significant changes in body weights, body weights of rabbits subjected to higher doses of IT SEB (1.0 to 3.0 mg/kg) decreased significantly ( $P < 0.05$ ) 20 hr after IT SEB. Decreased food and water intake were grossly observed without precise measurements. Changes in rectal temperatures after IT SEB administration were inconsistent among animals after an initial 20 hr elevation (Fig. 3). Elevated rectal temperatures returned to normal or decreased below normal values shortly before death.

During the course of IT SEB toxicosis in Dutch rabbits, predominant symptoms included inactivity and decreased response to human handling. Lethality was dose-dependent. The number of deaths for each administered dose of SEB is presented in Table 1. A sigmoid lethality curve after IT injections of SEB at doses ranging from 0.1 and 3.0 mg/kg, is illustrated in Fig. 4. Based on the data of lethality, an  $LD_{50}$  was determined to be 0.46 mg/kg ( $N = 30$ ) with 95% confidence limits (0.18 to 1.00 mg/kg). The percent of survival time lines after IT SEB challenge at different doses are presented in Fig. 5. There was a significant difference ( $P < 0.05$ ) on the curves between 0.3 and 3.0 mg/kg groups.



## Discussion

The high costs of SEB necessary to generate aerosol and a reluctance to induce illness and lethality in non-human primates led us to seek an alternative approach of SEB delivery to the lungs of a phylogenetically lower animal species. We selected Dutch rabbits because of plentiful supplies and our previous experience working with this specific animal species, using intramuscular injections of SEB (15, 16). The SEB-induced toxicity in rabbits (15, 16, 19, 20) and rhesus monkeys (6-11, 21) have been reported in the literature. Our main aims were to present data on SEB-toxemia associated fever, weight loss, and physical inactivity and also to show that IT SEB injected rabbits develop dose-related illness and death. To express more accurately what were learned from the lethality dose-response curve, we calculated  $LD_{50}$ , as well as high and low values at 95% confidence limits. We used limited numbers of rabbits ( $N = 30$  in 5 groups; approx. 6/group) in these studies.

Brain, et al. (22) described IT instillation for studying the pulmonary distribution of particles in ether-anesthetized hamsters and rats. The particle suspensions were delivered to the lung through an endotracheal approach via the mouth (22). In this study, however, we used direct IT injection of SEB to the Dutch rabbit under a short-acting gas anesthesia. The amount of administered SEB was precisely calculated as mg/kg body weight. No SEB was introduced directly into the digestive system. The IT method is unlike the aerosol approach in which more than 90% of

the applied toxin can be lost, lodging to the face, skin, hair, and in the digestive system, without reaching the animal's lungs. Furthermore, the magnitude of toxin loss depends on different designs of the aerosol exposure system (14, 23-26).

Although an equation presented by Guyton (27) has been used for estimating minute volume by using the body weight<sup>3/4</sup> (g)/1,000 x a constant (2.1) in several laboratory animals, there were no experiments to validate the Guyton's equation under aerosolization. Consequently, when the animal is subjected continuously to an environment of aerosol, one can not calculate the aerosol dose (mg or  $\mu$ g/kg) without knowing the animal's minute volume and percent deposition of aerosol in the respiratory tract (23, 24). Because the delivered aerosol dose to animals can not be determined quantitatively, it is difficult to establish a dose-response curve based upon animal weight of lethality by aerosolization. For physiological and pharmacological research which requires a quantitative assessment of dose delivered (mg or  $\mu$ g/kg body weight) versus the dosage in terms of air flow rate, solution concentration, and application time as used in aerosolization, IT instillation may be a preferred model.

To develop strategies for physiological support and pharmacological intervention of SEB toxicity of respiratory origin, it is essential to understand the kinetics or fate of SEB in the circulation and organ distribution. The use of direct IT injection of SEB appears simple and ideal for studying SEB kinetics, initiated from the lung. In the lung, SEB may be

transported into the interstitium from the bronchi or alveoli. While in the interstitial space, SEB may enter the systemic circulation via capillaries or the lymphatic system. Preliminary results from our currently ongoing research work support these unique concepts (unpublished observations).

Some differences exist in physiological and biochemical responses to IT and aerosol exposure of SEB (14, 19). We do not intend to replace aerosolization with IT injection, or exclusively substitute non-human primates with Dutch rabbits. Our key aim is to expand and verify a quantitative IT approach to introduce SEB into the lungs. Recognizing the problems of generating aerosols that are costly in equipment, safety facilities, and toxin consumption, a simple IT practice should save time and money.

In summary, 1) The Dutch rabbit is acceptable for studying SEB-induced respiratory and systemic toxicities. 2) The IT SEB approach is essential for studying pulmonary origin of SEB kinetics, and the obtained information will enhance our understanding of SEB-induced toxicity and diagnosis of SEB in plasma and tissues. 3) The IT SEB-instilled Dutch rabbit may be appropriate for evaluating potential immunological or/and pharmacological therapies of SEB toxicity induced from IT challenge via a pulmonary route

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### Figure Legends

- Fig. 1. Experimental set-up for direct intratracheal injection of SEB in the anesthetized Dutch rabbit.
- Fig. 2. Effect of intratracheal administration of SEB on body weight changes from baseline values in Dutch rabbits (\* $P < 0.05$  as compared to control group).
- Fig. 3. Effect of intratracheal administration of SEB on rectal temperature in Dutch rabbits.
- Fig. 4. The lethality curve after intratracheal injections of SEB at various doses in Dutch rabbits.
- Fig. 5. The percent of survival time lines after intratracheal injections of SEB at different doses in Dutch rabbits.

### Footnotes

<sup>1</sup> The opinion and assertions contained herein are the private views of the authors and not to be construed as official or as reflecting the views of the Department of Defense. In conducting the research described in this report, the authors adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the U. S. Department of Health and Human Services, NIH.

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<sup>3</sup> Address correspondence to: Ching-Tong Liu, Toxinology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21702-5011. Telephone (301)-619-2724; Fax: (301)-619-2348.

<sup>4</sup> Present address: Department of Medicine, Tripler Army Medical Center, Honolulu, HI 96859

Table 1. The lethality of Dutch rabbits after intratracheal instillation of SEB at various doses in Dutch rabbits

Dose (mg/kg)	Log dose	No. of animals	No. of deaths
0.00	----	4	0
0.10	-1.00000	5	0
0.30	-0.52288	8	4
1.00	0.0	9	6
3.00	0.47712	4	4



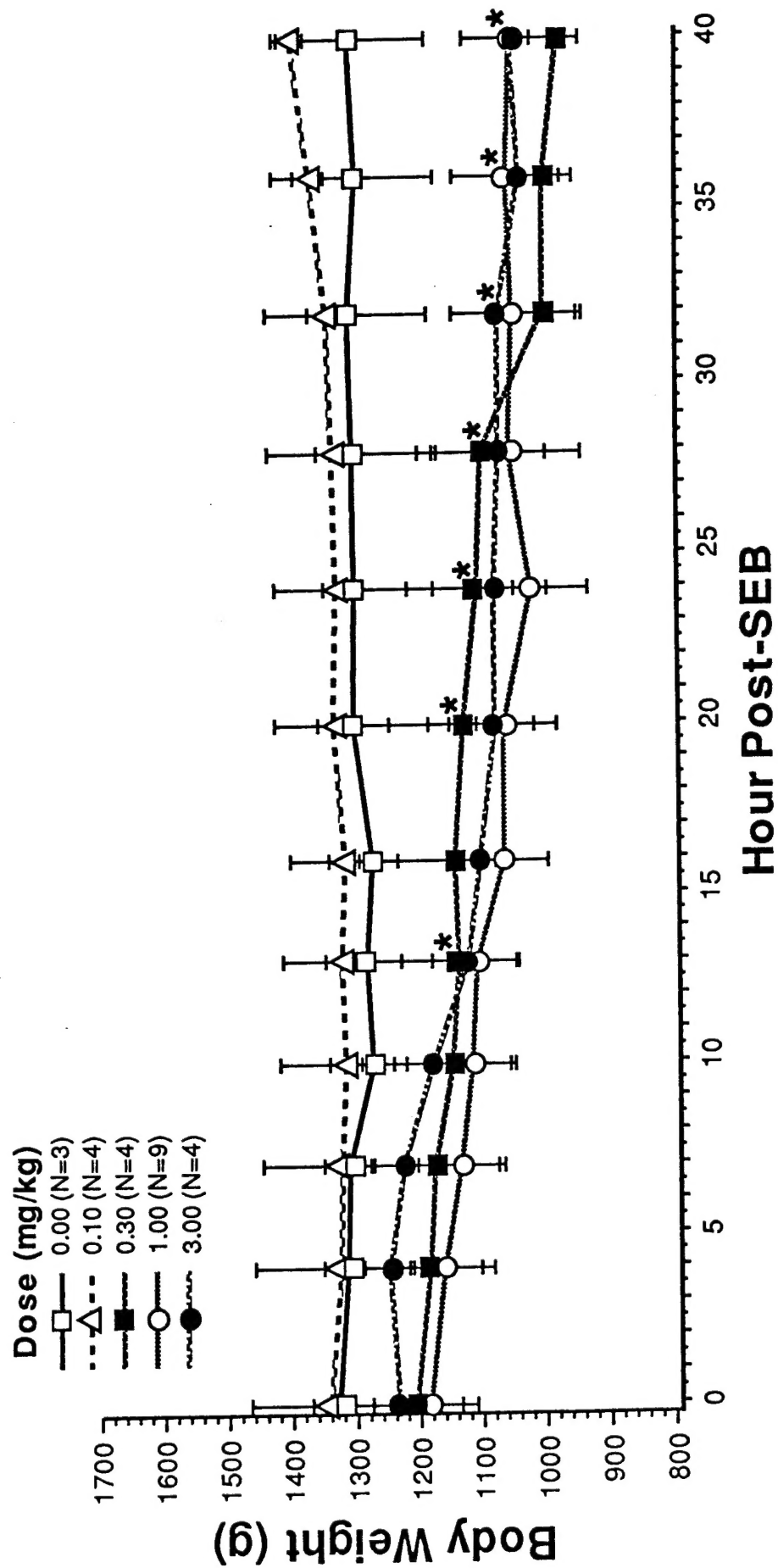


Fig. 2

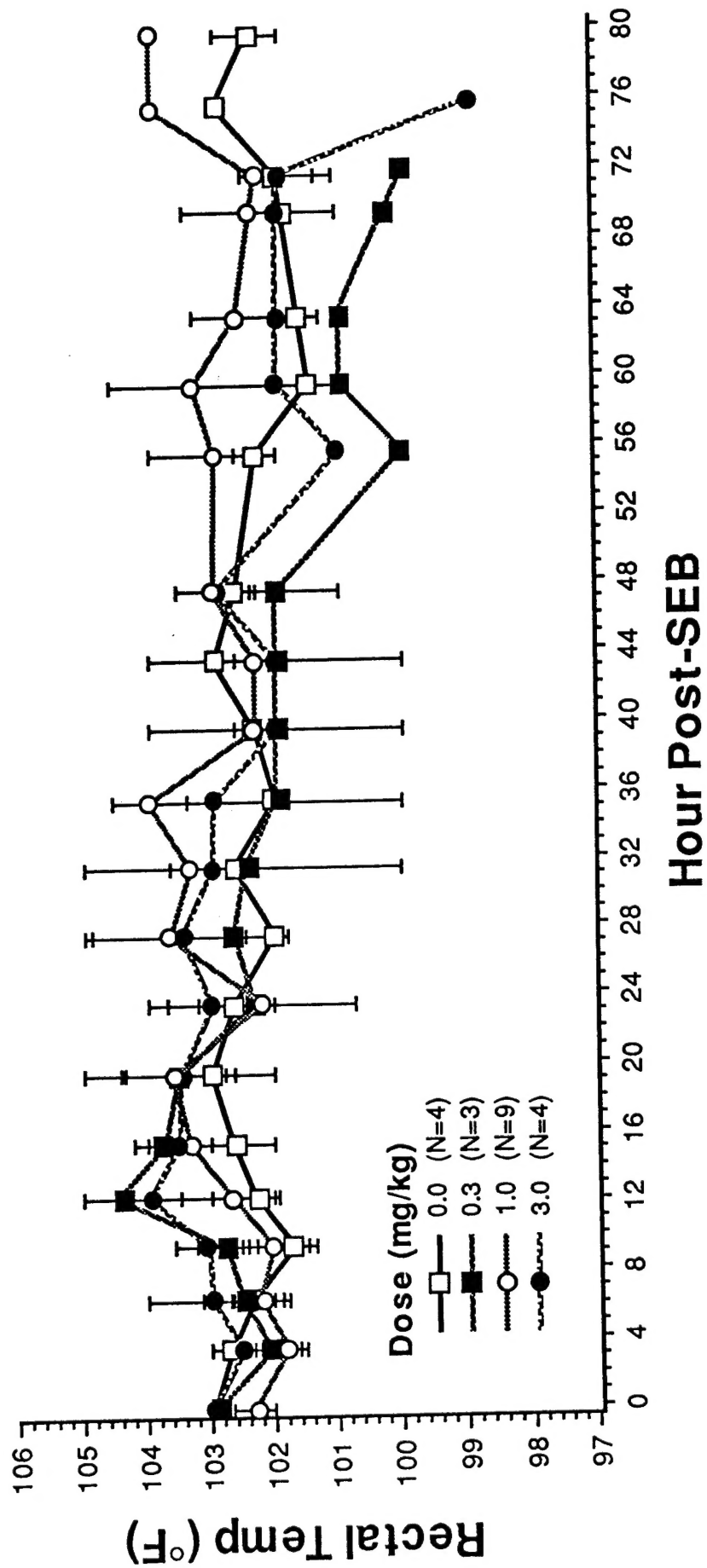


Fig. 3

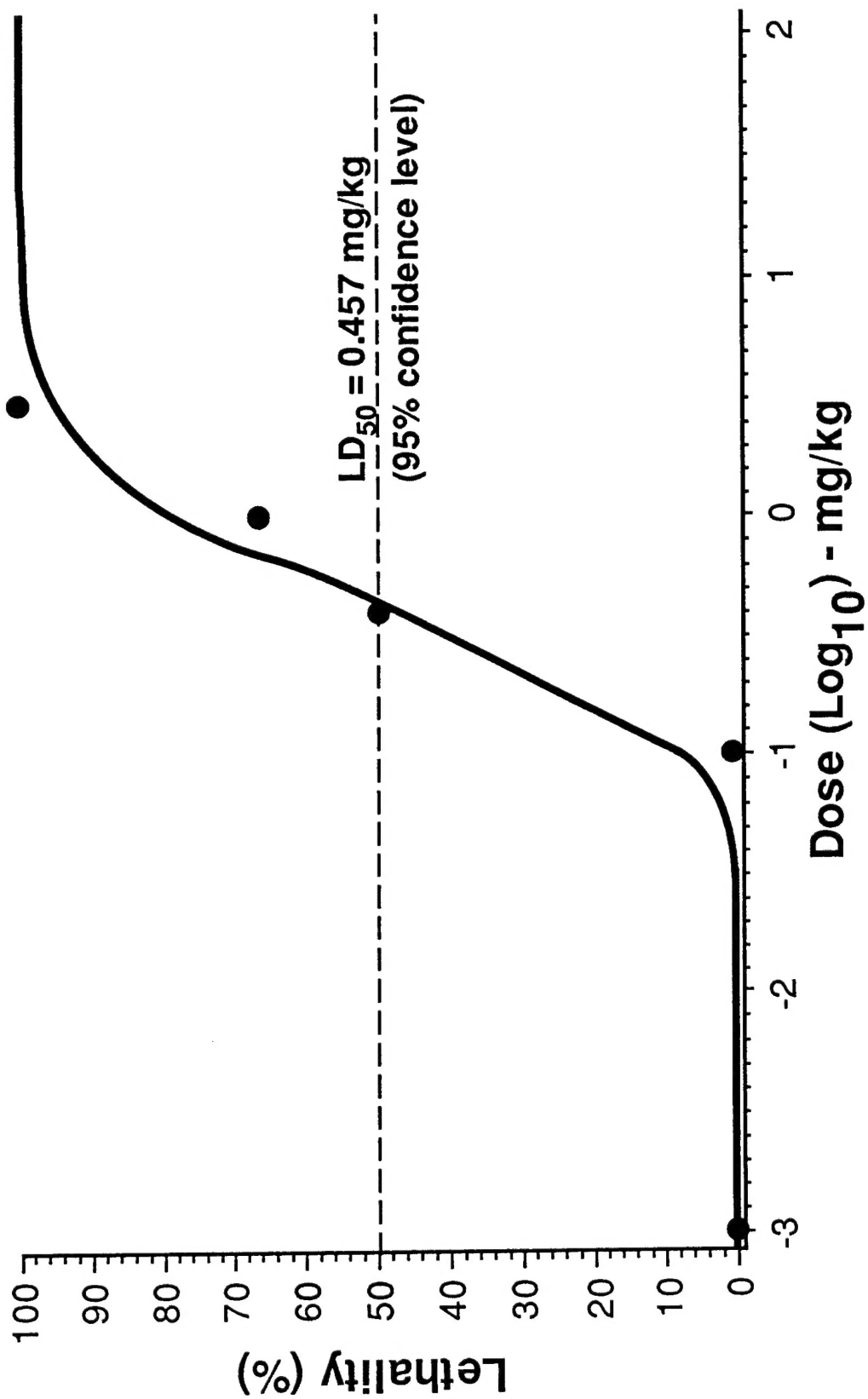


Fig. 4

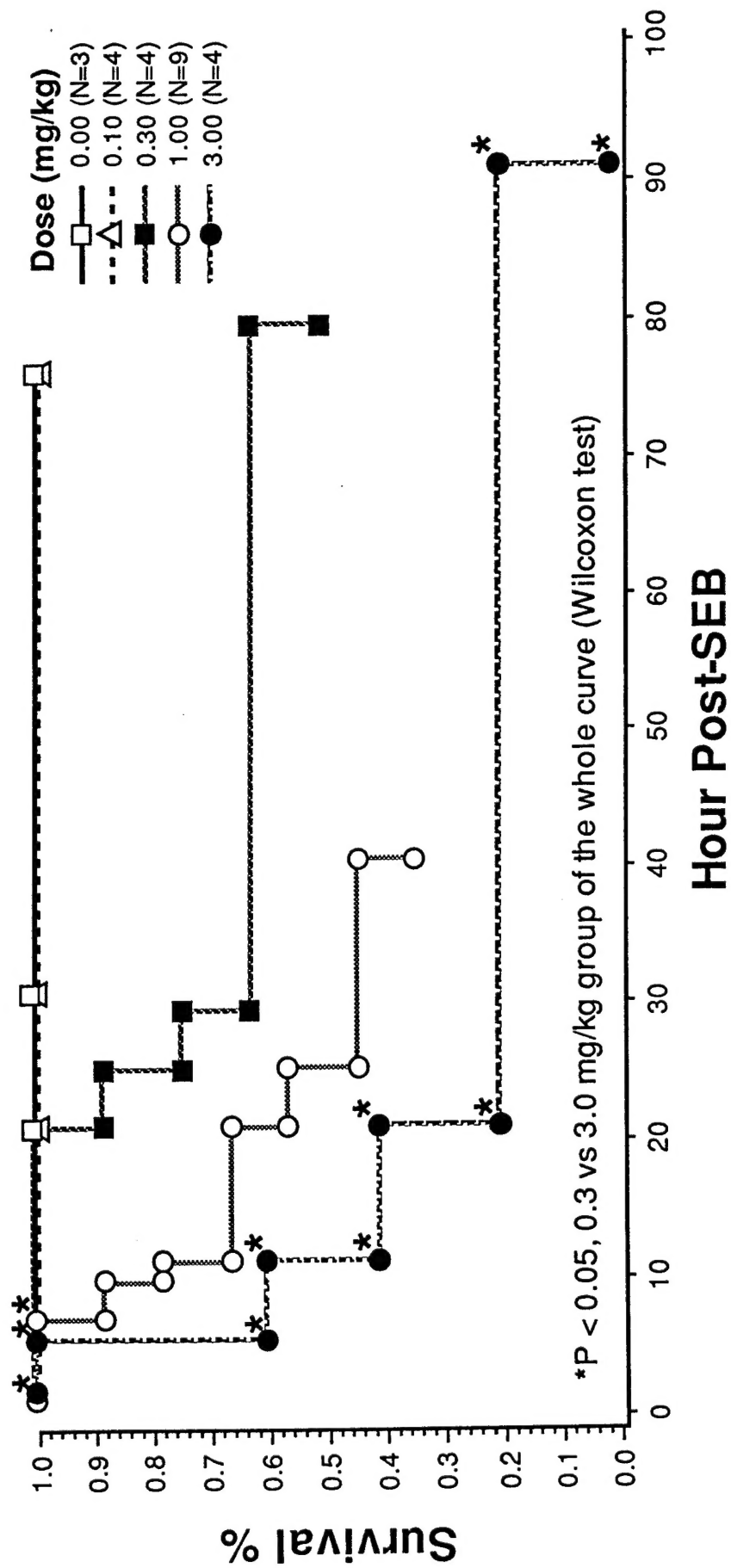


Fig. 5